CHANGES IN THE ULTRASTRUCTURE OF LAMB MUSCLES AS INFLUENCED BY FREEZING AND SODIUM TRIPOLYPHOSPHATE (STPP)

Abdel-Aal H. A., Mohamed H. M. A.

Department of Food Science, Faculty of Agriculture, Minia University, El-Minia, Egypt
e-mail hanimebeid@yahoo.com

Abstract

Three lamb muscles from shoulder, Supraspinatus (SS); lion, Longissimus dorsi (LD) and leg, Biceps femoris (BF) were kept in refrigerator at 4°C for 24 hr, dipped in 10% sodium tripolyphosphate (STPP) solution for 1 min and frozen at –18°C or at – 40°C for 48 hr. Proximate composition, phosphorus, drip loss, cooking loss, pH and water holding capacity (WHC) were determined. Ultrastructure of treated and untreated muscles was studied using transmission electron microscopy (TEM). No significant (p<0.05) differences were found between moisture, protein and fat content of the three lamb muscles. Muscles treated with STPP had higher phosphorus content than that of untreated muscles. Treated or untreated frozen lamb muscles at –40°C had lower drip losses and cooking losses than those frozen at –18°C. All three lamb muscles treated with STPP had lower drip losses and cooking losses and higher pH and WHC than those of untreated ones. Results of the study suggested that STPP increased the pH, improved the WHC and decreased drip loss and cooking loss of frozen lamb muscles. Freezing at –40°C and STPP treatment maintained the well-defined all sarcomere components of lamb muscles compared to untreated muscles and frozen at –18°C.

Key words: Lamb meat, water-holding capacity, sodium tripolyphosphate, transmission electron microscopy.

INTRODUCTION

Water retention is essential for meat product palatability attributes of juiciness and tenderness. In addition, water retention is economically important because loss of water results in a decreased amount of marketable product. Retention of water in meat is termed water-holding capacity (WHC), and the important of WHC to meat quality has been reviewed by (1) and (2).

Phosphates are widely used to improve binding in meat products (2), (3), (4), (5) and (6). However, the main reasons for adding polyphosphates in cured meat products and frozen whole meats are 1) to control the weep of natural juices that occurs from the time of slaughter to packaging and leads to a tough texture, reduced juiciness, and susceptibility to freezer burn. 2) Polyphosphates greatly reduce the loss of water on cooking, 3) they reduce the concentration of NaCl required for water uptake (7). When polyphosphates were used with NaCl, phosphates control fluid loss by increasing the water binding or the retention of the meat's natural juices. They accomplish this by increasing pH, increasing ionic strength, and complexing with protein-bond magnesium and calcium so that the actinomyosin muscle protein can dissociate, exposing more bonding sites for hydration (8). Sodium tripolyphosphates (STPP) are the most popular phosphate used in meat industry. It is accounting for 80% of the phosphates incorporated either as a single phosphate or in blends (9). Electron microscopy has been used to elucidate the effect of freezing, organic acids, salt, and polyphosphates treatments in the ultrastructure of meat muscles (7; 10 and 11). Relationship was found between changes in sarcomere length of bovine myofibril and cooking losses (12). The effect of salt and polyphosphate is to disrupt the sarcomere particularly in the myosin rich "A"- band region, protein is dispersed from the sarcomeres and this enables the meat to

retain more moisture and reduce cooking losses (13 and 14). Little attention has been paid to the ultrastructure changes in lamb meat during freezing. The objectives of this study were 1) to study the effect of freezing and STPP treatment on the drip loss, water holding capacity, and cooking loss of three lamb muscles. 2) to investigate ultrastructural changes in frozen three lamb muscles treated with STPP.

MATERIAL AND METHOD

Lamb carcasses averaged 21.5 kg dressed weight was obtained from Mallawi Experimental Farm, Animal Production Research Institute, Ministry of Agriculture, Egypt. Three muscles samples from shoulder, *supraspinatus* (SS); lion, *longissimus dorsi* (LD), and leg, *biceps femoris* (BF), were removed from carcasses within 1 hour post mortem, packed in Ziploc bags (Dow Brands L. P., Indianapolis, IN, USA) and kept in refrigerator at 4°C for 24 hr. Samples were cut into pieces of 125 g weight, 2.8 cm thick and 3.5 length and divided to two groups. The first group was used as a control and the second group was dipped in 10% sodium tripolyphosphate (STPP) solution for 1 min (2). One part of treated samples was kept at – 40 °C and the second part was kept at –18 °C for 48 hr.

Drip loss

Drip loss was determined in pieces of muscle according to the method described by Santos and Regenstein (15).

Proximate composition and phosphorus

Moisture, protein (Total N x 6.25), fat and ash were determined according to methods 934.01, 978.02, 920.39, and 938.08 (16). Phosphorus was measured in the dry ashed-extract by the colorimetric method (17).

pH

Meat samples (10 g) were homogenized in 90 ml distilled water. The pH of homogenized samples were measured using a glass pH electrode (ICM Digital pH, Model 41250, Hillsboro, OR, USA)

Water Holding Capacity (WHC)

Water Holding Capacity (WHC) was determined (18).

Cooking loss

A portion of meat was weighed, warped in aluminum foil and cooked in oven at 180°C. Meat samples were removed from the oven at 80± 3 °C. Internal temperature was monitored using a thermocouple (Omega, Model 199, Engineering Stamford, CN USA). Cook loss was determined by the difference in raw and cooked weights and reported as percent on initial meat weight (Lee, et al., 19).

Transmission Electron Microscopy

Small pieces of muscles (about 1mm^3) were fixed as soon as possible in 3% cacodylate buffered glutaraldehyde (pH 7.3), (20).

After two rinses in the buffer, the tissues were post fixed in 1% buffered osmium tetroxide, followed by washing twice in cacodylate buffer for 30 minutes.

The specimens were dehydrated in ascending grades of ethanol (50-100%), then cleared in toluene for 10 minutes and embedded in epoxy resin (21). Semi thin sections of 1.5 mμ were cut from each block and stained with toluidine blue for light microscopy examination. Ultrathin sections (700 A°) were cut with glass knives on a NOVA ultramicrotome. These sections were stained with saturated uranyl acetate in 70% alcohol for 20 minutes followed by lead acetate for 5 minutes. The sections were examined using a JEOL-100 CXII electron microscope.

Statistical analysis

Data were analyzed with the GLM (General Linear Model) program using Statistical analysis system (22). Mean values were compared by Duncan's Multiple Range Test.

RESULTS AND DISCUSSIONS

Moisture, protein, fat and ash content of the fresh three lamb muscles were presented in Table 1. No significant (p<0.05) differences were found between moisture, protein and fat content of the three lamb muscles. Ono et al (23) reported that moisture, protein fat and ash contents of six retail cuts of lamb were (75.23-72.04%), (21.04-18.72%), (7.35-2.89%) and (1.06-1.10%), respectively. *Supraspinatus* muscle

had significantly (p <0.05) lower ash content (1.13%) than that of *Longissimus dorsi* (1.15%) and *Biceps femoris* (1.5%) muscles.

Results in Table 2 revealed that treated or untreated frozen lamb muscles at –40°C had lower drip losses and cooking losses than those frozen at –18 °C. The amount of drip losses increased in post mortem meat due to decreasing the pH or slow freezing (7). Slow freezing (-10°C) generated fewer large ice crystals which distort cells and crushed myofibrils (24). All three lamb muscles treated with STPP had lower drip losses and cooking loses than those of untreated. This may be attributed to the STPP which increased the pH and increased the water retention of meat (7 and 3). Polyphosphates enhance the solubilisation of myosin and reduce cooking losses, probably by forming a myosin gel fine cover or membrane which traps the water (14 and 25).

Phosphorus contents of untreated three lamb muscles were ranged from 147.43 mg/100g to 233.84 mg/100g. *Longissimus dorsi* (LD) contained the higher phosphorus levels compared to other muscles. Data from this study are consistent with previous findings (22 and 23). Phosphorus levels and ash content of treated lamb muscles were increased (Table 2). This is possibly due to the penetration of STPP into muscles during treatment. STPP increased the water-holding capacity of lamb muscles frozen at –18 °C or at –40 °C (Table 3). Treated or untreated *Biceps femoris* (BF) had the highest values of WHC. Polyphosphates improve the water-holding capacity of raw meat by increasing the pH value from the isoelectric point (about pH 5.5) towards the alkaline side and their ability to dissociate actomyosin into actin and myosin of meat (1, 25, 27 and 7).

Table 1. Proximate composition of three lamb muscles Supraspinatus (SS), Longissimus dorsi (LD) and Biceps femoris (BF)

Components %	*Shoulder* [Supraspinatus (SS)]	Lion [*Longissimus dorsi (LD)*]	Leg [*Biceps femoris (BF)*]
Moisture	75.88a ±0.23	75.34a ±0.13	75.20a ±0.76
Protein	19.16a ±0.49	19.29a ±0.77	19.43a ±0.50
Fat	3.42a ±0.17	3.58a ±0.32	3.63a ±0.70
Ash	1.13b ±0.01	1.15a ±0.05	1.15a ±0.02

a b Mean values in same row not followed by the same letter are significantly different (P <0.05). Means of triplicates ±SD

Table 2: Drip loss, cooking loss and pH of three lamb muscles treated with STPP and frozen at –18 °C and –40 °C.

Samples	Drip loss %	Cooking Loss %	Ash %	Phosphorus (mg/100g)	PH
-18 °C untreated					
Supraspinatus (SS)	4.82	39.36	1.13	166.86	6.40
Longissimus dorsi (LD)	4.92	35.55	1.10	233.84	6.30
Biceps femoris (BF)	5.53	42.55	1.13	147.43	6.00
-18 °C treated with STPP					
Supraspinatus (SS)	2.46	34.50	1.47	197.08	6.60
Longissimus dorsi (LD)	3.06	32.73	1.37	266.09	6.60
Biceps femoris (BF)	4.65	37.00	1.32	160.65	6.65
-40 °C untreated					
Supraspinatus (SS)	3.33	36.27	1.13	150.40	6.15
Longissimus dorsi (LD)	3.16	33.35	1.20	226.69	6.60
Biceps femoris (BF)	3.17	40.38	1.16	154.02	6.15
-40°C treated with STPP					
Supraspinatus (SS)	2.04	33.80	1.39	186.78	6.45
Longissimus dorsi (LD)	2.11	32.50	1.69	268.90	6.70
Biceps femoris (BF)	2.28	34.50	1.37	169.93	6.35

Means of two replicates

Table 3: Moisture, water holding capacity, ash, and phosphorus content of three lamb muscles treated with STPP and frozen at –18 °C and –40 °C.

Samples	Moisture %	Water Holding Capacity (WHC)
-18 °C untreated		
Supraspinatus (SS)	74.43[c] ±0.56	36.17[j] ±1.92
Longissimus dorsi (LD)	74.53[b] ±0.16	35.73[k] ±0.53
Biceps femoris (BF)	73.95[g] ±0.01	37.32[g] ±1.10
-18 °C treated with STPP		
Supraspinatus (SS)	74.31[d] ±1.07	37.99[f] ±1.81
Longissimus dorsi (LD)	74.05[f] ±0.50	36.53[i] ±0.32
Biceps femoris (BF)	74.71[a] ±0.42	39.12c ±2.09
-40 °C untreated		
Supraspinatus (SS)	74.19[e] ±1.06	37.01[h] ±1.26
Longissimus dorsi (LD)	74.65[a] ±0.48	38.25[e] ±0.95
Biceps femoris (BF)	73.84[h] ±0.09	38.24[e] ±0.38
-40°C treated with STPP		
Supraspinatus (SS)	74.23d[e] ±0.50	38.55[d] ±0.96
Longissimus dorsi (LD)	74.40[c] ±0.51	39.81[b] ±1.96
Biceps femoris (BF)	74.31[d] ±0.22	41.05[a] ±1.80

[abcdefghi] Mean values in same column not followed by the same letter are significantly different (P< 0.05).   Means of triplicates ± SD

Ultrastructure

Fig 1.1, 2.1 and 3.1 presented the electron micrographs of longitudinal section of pre-rigor unfrozen *Biceps femoris* (BF), *Supraspinatus* (SS) and *Longissimus dorsi* (LD) lamb muscles. The micrographs represent myofibril showing characteristic regular appearance of the striated skeletal muscle. The myofibrils are surrounded by thin sheaths of sarcoplasm. The essential features of a sarcomers (S) are clearly shown. Myosin rich (a dens) A-bands (A), Actin rich (a light) I-bands (I), a lighter H-zone (H) with its more dens M-line (M) lie between two Z-lines. In general, all these electron micrographs exhibited clear definition of all sarcomere components of the (BF), (SS) and (LD) skeletal muscles fixed in pre-rigor stage (22 and 11).

Fig. 1.2, 2.2 and 3.2 revealed that electron micrographs of treated muscles frozen at – 40 °C showed clear definition of all components of the sarcomeres as the same of unfrozen muscles fixed in pre-rigor stage except slight loss in the H-zone of (LD) (Fig. 3.2). These muscles also, had well-defined Z-lines and I-band. (8, 29) reported that meat treated with 0.5% phosphate showed normal structure.

Fig. 1.3, 2.3, and 3.3 showed that untreated muscles frozen at – 40 °C had less definable H-zone and M-line. The Z-lines were also partially disorganized and fragmentation of the thin filaments in the region of the Z-lines was also apparent.

Generally, frozen untreated muscles at – 40°C had better clear definition of all sarcomere components than those frozen at – 18°C. These results agreed with those reported for fish muscle frozen slowly in a refrigerator or quickly in liquid nitrogen (24).

Fig. 1.4, 2.4, and 3.4 indicated that treated muscles (LD), (BF) and (SS) frozen at –18°C had definable H-zones and M-lines. The Z-lines were not completely damaged and the myofibrils still maintain their typical configuration of banding pattern. Lewis (13) reported that the myofibrillar proteins within the sarcomeres of meat treated with polyphosphate become more clearly defined.

However, freezing untreated muscles at – 18 °C caused changes and created distortion in the regular arrangement of the sarcomere component. The H-zones and its M-lines are

difficult to distinguish in all untreated (BF, SS and LD) muscles There was also some loss of the Z-lines and the I-band (Fig. 1.5, 2.5, and 3.5).
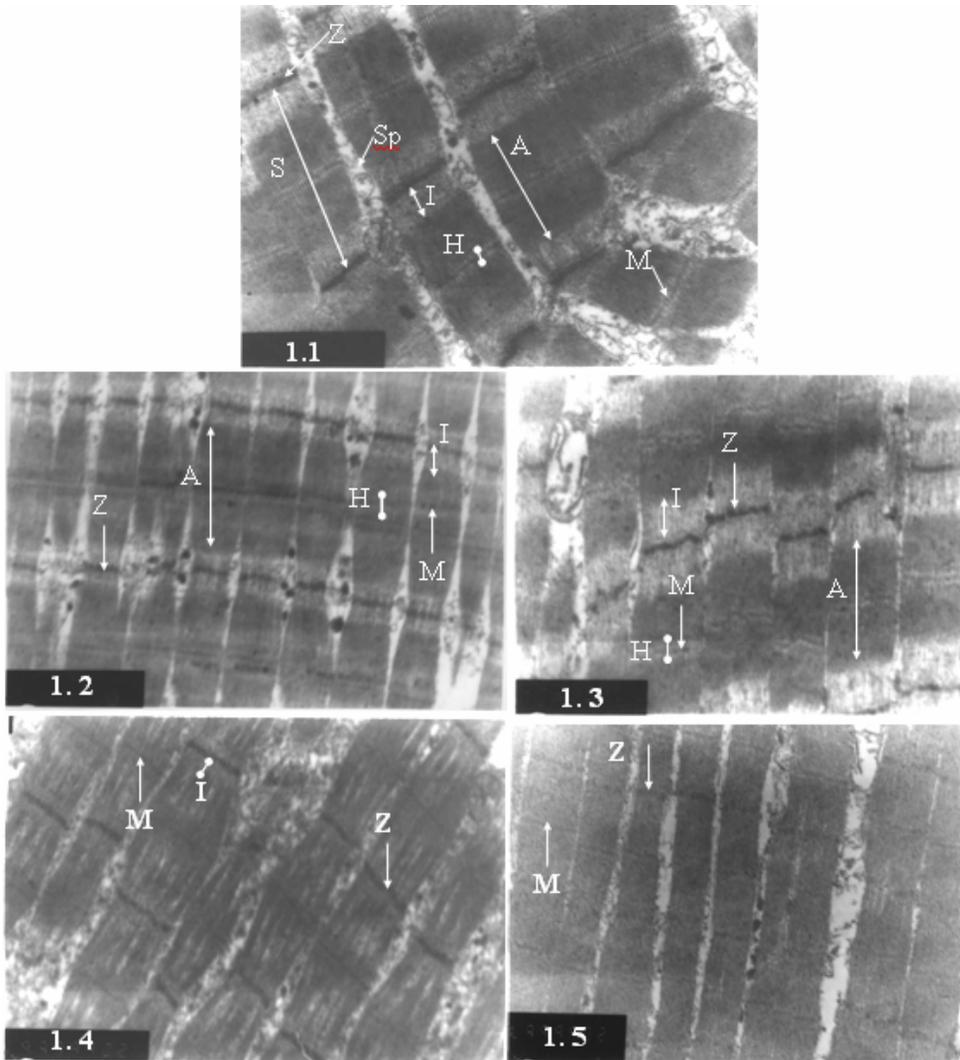

Fig. 1- Electron micrographs in longitudinal section of lamb Biceps femoris (BF) muscle (20000 x).
1.1- Unfrozen muscle.   1.2-Muscle frozen at-40° and treated with STPP
1.3- Muscle frozen at -40°C and untreated with STPP
1.4- Muscle frozen at-18°C and treated with STPP
1.5- Muscle frozen at-18°C and untreated with STPP
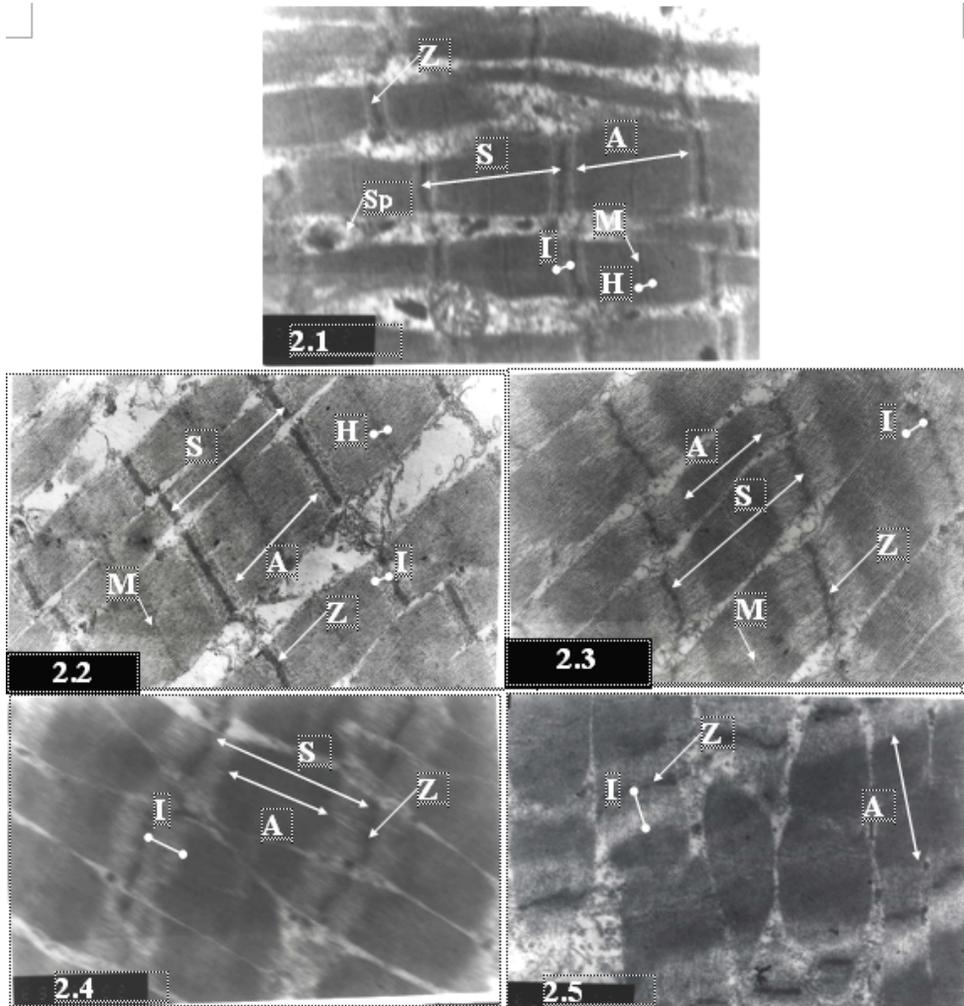A = A- band; H = H-zone; M = M-line; I= I band; S= Sarcomere; Sp=Sarcoplasm.

Fig. 2- Electron micrographs in longitudinal section of lamb supraspinatus (SS) muscle (20000 x).
2.1- Unfrozen muscle.   2.2-Muscle frozen at-40° and treated with STPP
2.3- Muscle frozen at -40°C and untreated with STPP
2.4- Muscle frozen at-18°C and treated with STPP
2.5- Muscle frozen at-18°C and untreated with STPP
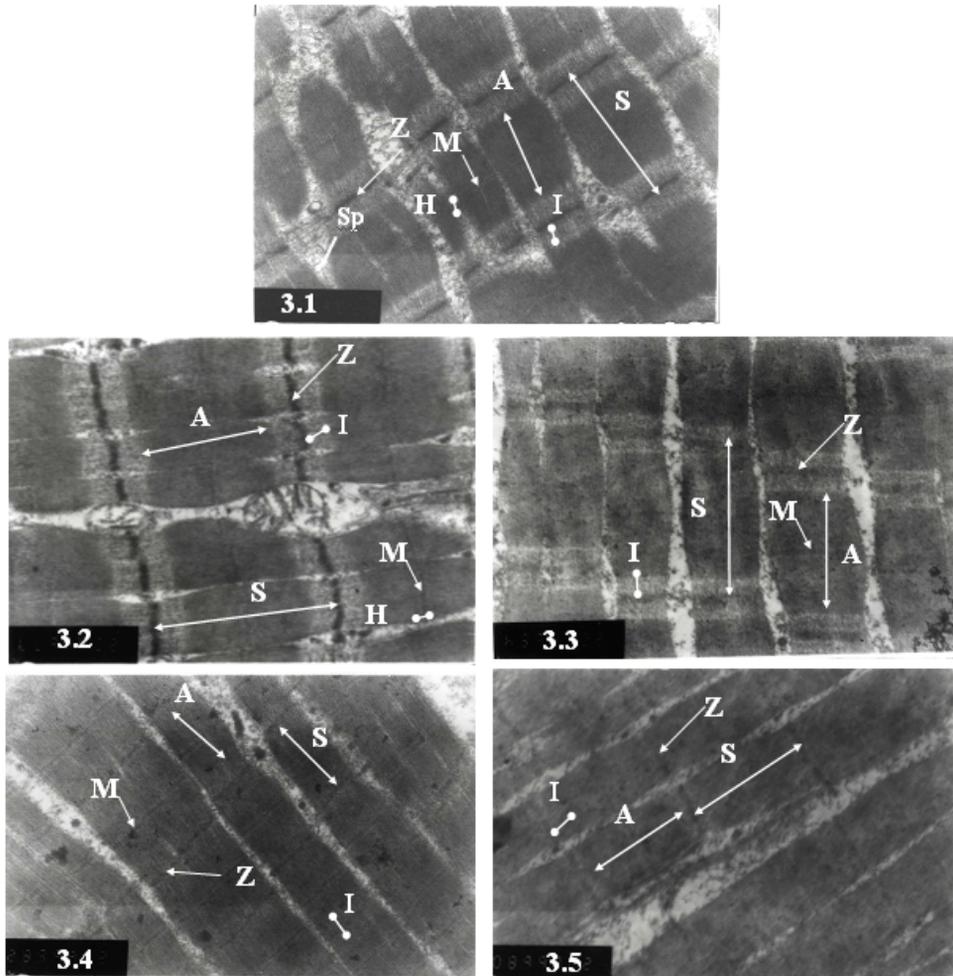A = A- band; H = H-zone; M = M-line; I= I band; S= Sarcomere; Sp=Sarcoplasm.

Fig. 3- Electron micrographs in longitudinal section of lamb Longissimus dorsi (LD) muscle (20000 x).
3.1- Unfrozen muscle.   3.2-Muscle frozen at-40° and treated with STPP
3.3- Muscle frozen at -40°C and untreated with STPP
3.4- Muscle frozen at-18°C and treated with STPP
3.5- Muscle frozen at-18°C and untreated with STPP
A = A- band; H = H-zone; M = M-line; I= I band; S= Sarcomere; Sp=Sarcoplasm.

CONCLUSIONS

STPP increased the pH values, improved the water retention and decreased drip loss and cooking loss of frozen lamb muscles. *Longissimus dorsi* (LD) muscle treated with STPP and frozen at –40°C had the highest values of pH, phosphorous and the lowest cooking loss. Freezing at –40°C and STPP treatment maintained the well-defined all sarcomere components of lamb muscles compare to untreated muscles and frozen at –18 °C.

REFERENCES

[1] Hamm, R. (1971). Interactions between phosphates and meat proteins. In "Phosphates in food processing," Ed. Deman, J. M., and Melnychn, P., Chapt. 5, pp. 65-82, AVI Publishing Co., Westport, CT.
[2] Molins, R. A. (1991). Phosphates in Food. CRC press, Boca Raton, Florida, USA.

[3] Dziezak, J. D. (1990). Phosphates improve many foods. Food Technol., 44 (4): 80-92.

[4] Panda, S. K., Singh, R. P., Anand, S. K. (1995). Effect of polyphosphate chilling and packaging on the quality of fried quail stored in refrigerator. Journal of Food Science and Technology, 32 (2): 156-158.

[5] Moiseev, I.V. and Cornforth, D.P. (1997). Sodium hydroxide and sodium tripolyphosphate effects on bind strength and sensory characteristics of restructured beef rolls. Meat-Science; 45 (1) 53-60.

[6] Sutton, D. S., Brewer, M. S., McKeith, F.K., (1997). Effects of sodium lactate and sodium phosphate on the physical and sensory characteristics of pumped pork loins. Journal of Muscle Foods; 8 (1): 95-104.

[7] Offer, G., Kinght, P., Jeacocke, R., Almond, R., Cousins, T., Elsey, J., Parsons, N., Sharp, A., Starr, R., and Purslow, P. (1989). The structural basis of water-holding, appearance and toughness of meat and meat products. Food Microstructure 8:151-170.

[8] Wong, D. W. S. (1989). Additives. Ch. 9 in "Mechanism and theory in food chemistry", p. 314 Van Nostrand Reihold, New York, USA.

[9] Barbut, S., Maurer, A. J. and Lindsay, R. C. (1988). Effects of reduced sodium chloride and added phosphates on physical and sensory properties of turkey frankfurter. J. Food Sci., 53 (1): 62-66.

[10] Rao, M. V., Gault, N. F. S., and Kennedy, S. (1989). Changes in the ultrastructure of beef muscle as influenced by acidic conditions below the ultimate pH. Food Microstructure, 8: 115-142.

[11] Mikel, W. B., Goddard, B. L., and Bradford, D. D. (1996). Muscle microstructure and sensory attributes of organic acid-treated beef strip lion. J. Food Sci., 61 (5): 1058-1061, 1093.

[12] Palka, k., and Daun, H. (1999). Changes in texture, cooking losses, and myofibrillar structure of bovine M. semitendinosus during heating. Meat Science, 51: 237-243.

[13] Lewis, D. F. (1981). The use of microscopy to explain the behavior of food-stuffs. A review of work carried out at the Leatherhead Food Research Association. Scanning Electron Microscopy. III: 391-404.

[14] Velinov, P. U., Zhikov, M. V., and Cassens, R. G. (1990). The effect of tumbling, sodium chloride and polyphosphates on the microstructure and appearance of whole-muscle processed meats. Food Microstructure, 9: 91-95.

[15] Santos, E. E. M. and Regenstein, J. M. (1990). Effects of vacuum packaging, glazing, and erythorbic acid on the shelf-life of frozen white hake and mackerel. J. Food Sci., 55: 64-70.

[16] AOAC, (1995). Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists International, Arlington, Virginia, USA.

[17] Fiske, C. H., and Subbarow, Y. (1925). The calorimetric determination of phosphorus. J. Biol. Chem. 66: 375.

[18] Alvarez, C., Couso, I., Solas, M.T., and Tejada, M. (1992). Influence ofmanufacturing process conditions on gels made from sardine surimi. In "Food Proteins structure and Functionality" K.D. Schwenke and R. Mothes (Eds.), pp. 347-353. VCH Verlagesellschaft, Germany.

[19] Lee, B. J., Hendrick, D. G., and Conforth, D. P. (1998). Effect of sodium phytate, sodium polyphosphate and sodium tripolyphosphate on physico-chemical characteristics of restructured beef. Meat Sci., 50 (3): 273-283.

[20] Sabatini, D. D., Bensch, K. C., and Barrnet, R. J. (1963). Cytochemistry and electron microscopy- the preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J Cell Biol., 17: 19-58.

[21] Luft, J. H. (1961). Improvements in epoxy resin embedding methods. J. Biophysic. Biochem. Cytol., 9: 409-414.

[22] SAS (1987). SAS/STAT Guide for personal computers, Version 6 ed., SAS Institute, Cary, NC, USA.

[23] Ono, K., Berry, B. W., Johnson, H. K., Russek, E., Parker, C. F., Cahill, V. R., and Althouse, P. G. (1984). Nutrient composition of lamb of two age groups. J. Food Sci., 49 (5): 1233-1239, 1257.

[24] Bello, R. A., Luft, J. H., and Pigott, G. M. (1982). Ultrastructural study of skeletal fish muscle after freezing at different rates. J. Food Sci., 47 (7):1389-1394.

[25] Zhao,Y., and Sebranek, J. G. (1996). Physicochemical and sensory qualities of fresh pork chops as affected by phosphate, ascorbate or sorbate dipping prior to irradiation. J. Food Sci., 61 (6): 1281-1284.

[26] Lin, K. C., and Cross, H. R. (1989). Mineral composition of lamb carcasses from the United States and New Zealand. Meat Science 24: 47-59.

[27] Shults, G. W., Russell, D. R., and Wierbicki, E. (1972). Effect of condensed phosphates on pH, swelling and water-holding capacity of beef. J. Food Sci., 37 (6): 860-864.

[28] Trout, G. R., and Schmidt, G. R. (1986). Effect of phosphates on the functional properties of restructured beef rolls: The role of pH, ionic strength, and phosphate type. J. Food Sci., 51 (6): 1416-1423.

[29] Theno, D.M., Siegel, D. G., and Schmidt, G. R. (1978). Meat massaging: Effects of salt and phosphate on the ultrastructure of cured porcine muscle. J. Food Sci., 43 (2): 488-492.